Supporting Information for

Lectin inspired polymers based on dipeptide Ser-Asp for glycopeptide enrichment

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Experimental

Materials and reagents

Bovine fetuin, bovine serum albumin (BSA), trypsin from bovine pancreas (TPCK-trypsin), PNGase F, dithiothreitol (DTT), iodoacetamide (IAA), ammonium bicarbonate (NH_4HCO_3), Formic acid (FA), urea, trifluoroacetic acid (TFA), L-arginine, N-acetyl-neuraminic acid (Neu5Ac), phenylmethanesulfonyl fluoride (PMSF) and ZIC-HILIC materials were ordered from Sigma-Aldrich (St. Louis, MO, USA). Ammonium formate ($HCOONH_4$) and HPLC-grade Acetonitrile (CH_3CN) were obtained from Merck (Darm-stadt, Germany). GELoader tips were products of Eppendorf (Hamburg, Germany). Milli-Q water (Millipore, Bedford, MA, USA) was used in all experimental processes.

Amino-modified silica gel (particle size, 5.0 μm; pore diameter, 30 nm; specific surface area, 120 m^2/g) was ordered from Fuji Silysia Chemical LTD (Kasugai Aichi, Japan). Acrylamide Ser-Asp was ordered from Synpeptide Corp. (Shanghai, China) with high purity (>95%). Acryloyl chloride, nitric acid, methanol (CH_3OH), trifluoroacetic acid (TFA), ethanol, acetonitrile (ACN), *N*, *N*'-dimethyl formamide (DMF), bromoisobutyryl bromide, and *N*, *N, N', N', N*"-pentamethyldiethyl-enetriamine were used as received. Copper bromide (CuBr) with a purity of 99.999% was recrystallized before being used. Toluene, chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) were dried by molecular sieves for 24 h before use. Double distilled water (18.2 MΩ·cm, MilliQ system, Bedford, MA, USA) was used. Diverse disaccharides and fructo-oligosaccharides were purchased from TCI Corp. (Japan) with high purities (>99%).

Instruments:

Brunauer-Emmet-Teller (BET) adsorption isotherm curves were obtained on a on a Quadrasorb SI (Quantachrome, USA). The pore diameter was calculated based on the Barrett-Joyner-Halenda (BJH) method. Peptides derived from standard protein were analyzed with a nano electrospray ionization-quadrupole time-of-flight mass spectrometer (ESI-Q-TOF MS) (Waters, Manchester, UK). Peptides resulting from real biosamples were separated and characterized with Accela 600 high performance liquid chromatography (HPLC) coupled with LTQ-Orbitrap Velos mass spectrometer (Thermo Corp., CA, USA). Zeta potential was measured on a Malvern Zetasizer 2000/3000 instrument. Static contact angles were measured by a contact angle measurement system (Dataphysics OCA35 instrument) at ambient atmosphere and a constant temperature of 20 ^{o}C. High resolution scanning electron microscopy (HMSEM) spectra were recorded on a Hitachi S-4800 SEM (Hitachi Corp., Toyko, Japan).

Preparation of Ser-Asp modified silica gels.

The amino-modified silica gels (3.0 g) were suspended in 30 mL of anhydrous CH_2Cl_2 with pyridine. The polymerization initiator bromoisobutyryl bromide (BIBB, 4.0 mL) was added dropwise to this solution for 20 min at 0 ^{o}C and stir overnight at ambient temperature. The resulting product was obtained by centrifugation at 8000 rpm for 10 min and washed thoroughly with CH_2Cl_2. Acrylamide Ser-Asp was grafted onto silica gels through a surface initiated atom transfer radical polymerization. Briefly，acrylamide Ser-Asp (2.0 g) was dissolved in 20 mL distilled water and mixed with the BIBB-modified silica gels. The solution was mixed with copper bromide (CuBr, 0.064 g, 0.46 mM) and pentamethyldiethylenetriamine (PMDETA, 0.32 mL). The solution was stirred for 6 h at 60 ^{o}C and the product was separated by centrifugation at 8000 rpm for 10 min. The resulting polymer-modified silica gels were washed with purified water and then ethanol. Thus PEI-*g*-SD modified silica gels (PolySD-SiO_2) was obtained and dried under vacuum.

Chromatographic separation of disaccharide and fruto-oligosaccharides.

PolySD-SiO_2 (2.5 g) was suspended in 90 mL of CH_3OH and packed into a stainless steel column (250 mm×4.6 mm) under 40 MPa. Disaccharides and fruto-oligosaccharides were used to evaluate the saccharide separation effects by PolySD-SiO_2 packed columns. This evaluation experiments were performed on an Alliance HPLC system equipped with a Waters 2695 HPLC pump, a Waters 2424 evaporative light scattering detection (ELSD) system (Waters, Milford, MA, USA). The flow rate was 1.0 mL min^{-1} and the column temperature was 30 ^oC. The mobile phase A: 100 % CH_3CN，the mobile phase B: 100 % H_2O. Gradient for separation of disaccharides: 0-12 min, 80%-65% A; Gradient for separation of fruto-oligosaccharides：0 - 12 min, 80% - 65% A; 12 - 27 min, 65%-55% A. ELSD condition: gas pressure 30 psi, tube temperature 70 ˚C, gain 10.

Protein digestion

HeLa S3 cell were treated with sonication. The resulting solution was transferred to centrifuge tube and centrifuged at 15,000 rpm for 30 min at 4°C. The supernatants were collected protein concentration was determined by ultraviolet-visible photometer.

Proteins were denatured in urea (8 M)/NH_4HCO_3 solution (25 mM), reduced by DTT for 45 min at 56 °C and subsequently carboxymethylated with IAA in the dark for additional 30 min at room temperature. Then the mixture was diluted to tenfold volume with NH_4HCO_3 (50 mM) buffer. Finally, trypsin was added at an enzyme/substrate ratio of 1:20 (w/w) into the diluted solution and incubated for 16 h at 37 °C. The reaction was quenched by FA at the final concentration of 1%.

Enrichment of glycopeptides with PolySD-SiO_2

PolySD-SiO_2 material (2mg) were washed and equilibrated with 40μL of 40% CH_3CN/H_2O/5 mM NH_4HCO_3 and 80 μL of 80% CH_3CN/H_2O/0.1% FA under dispersive solid phase extraction (dSPE) mode. Tryptic fetuin digests were mixed with desalted BSA digests (at different molar ratios) in 80% CH_3CN/H_2O/0.1% FA. After incubation peptide mixture with PolySD-SiO_2 for 30 minutes with gentle

rotation, the tube was centrifuged for 3 mins at 3000 rpm. The remaining materials and peptides were washed with 100 μL 70% CH_3CN/H_2O/0.1% FA for 3 times after centrifugation. Glycopeptides were finally eluted from PolySD-SiO_2 with 20 μL 40% CH_3CN/H_2O/5 mM NH_4HCO_3.With the increased amount of BSA interference, the material amount and wash volumes were increased accordingly.

Concerning the capture of glycopeptides from tryptic digests of HeLa S3 cell lysate, similar protocol was followed. The enriched glycopeptides were dried with speed vacuum and redissolved with 40 μL 25 mM NH_4HCO_3. Removal of glycan moiety was performed with 1 unit of PNGase F at 37 °C overnight.

Recovery rate analysis

A standard glycopeptide with sequence KVA*N*KT (*N*: glycosylation site) was used to investigate the recovery of the established approach. The procedure of stable isotope labeling dimethyl on glycopeptide was carried out according to literature[1]. The light labeled glycopeptide was detected as m/z 983.7893(3+) and heavy labeled one was identified at m/z 987.7397(3+) in the MS. Light-labeled glycopeptide was treated with PolySD-SiO_2. After enrichment, the eluted light-labeled glycopeptide was mixed with the same amount of heavy-labeled SG and the mixture was infused into MS. The recovery of glycopeptide was calculated according to the relative intensities of light- and heavy- labeled ones.

Mass spectrometer analysis and database search

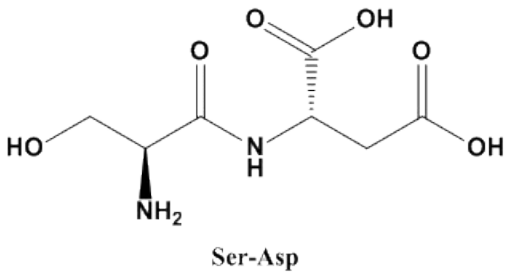
The glycopeptides enriched from tryptic digests of standard proteins were analyzed with a nano electrospray ionization-quadrupole time-of-flight MS (ESI Q-TOF MS, Waters, and Milford, MA, USA). The peptides were directly infused into ESI source with Nano Acquity UPLC (Waters, Milford, MA, USA). Positive full scan mode was used to acquire data. The full scan was performed from m/z 600 to 2000 and tandem mass scan mode was recorded from m/z 100-2000, respectively.

For the case of glycopeptides captured from HeLa S3 cell lysate, the peptides were firstly treated with PNGase F. The resulting deglycosylated peptides were separated with nano LC column. Before separation with nano LC column, the peptides were firstly loaded onto C18 trap column (200 μm×40 mm, 5 μm, 120 Å)

and separated with home-made nano column (75 μm×120 mm, 3 μm, 120 Å). Two mobile phases were used and they were 0.1% FA in water (A) and 0.1% FA in CH_3CN (B). A 100 minute gradient elution was used with 5–22% B in 55 minutes, 22–35% B in 25 minutes, 35–80% B in 10 minutes, 80% B in 10 minutes. The separated deglycosylated peptides were further characterized with LTQ-Orbitrap Velos coupled with Accela 600 HPLC system (Thermo, San Jose, CA). Full scan mode were performed at the mass range from m/z 400 to 2000 (R = 60,000 at m/z 400). The 10 most intense ions from the full scan were selected for collision induced dissociation (CID) in the ion trap. The dynamic exclusion function was set as follows: repeat count 1, repeat duration 30 s, and exclusion duration of 60 s.

The RAW files collected by Xcalibur 2.1 were converted to *.MGF by Proteome Discoverer (v1.2.0.208, Thermo, San Jose, CA) and searched with Maxquant[2] (version 1.3.0.5, Max Planck Institute of Biochemistry, Martinsried, Germany) using Uniprot protein fast database of mouse. Cysteine carboxamidomethylation (C) was set as a fixed modification, oxidation on methionine (M) and deamidation (NQ) were set as the variable modifications. Up to two missing cleavages of trypsin were allowed. Mass tolerances were set as 10 ppm and 0.1 Da for the parent and fragment ions, respectively. A false discovery rate (FDR) of 1% was set for both peptide identification and glycosylation site analysis.

Schemes



Ser-Asp

Scheme S1. Chemical structures of Ser-Asp

Figures

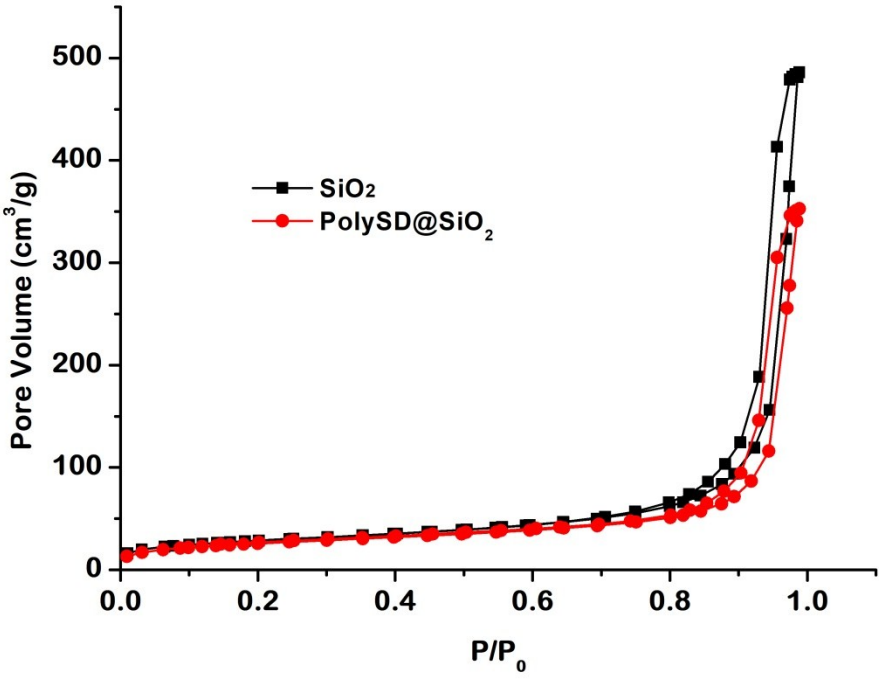


Fig. S1. Nitrogen adsorption–desorption isotherm plots of PolySD-SiO_2 and SiO_2.

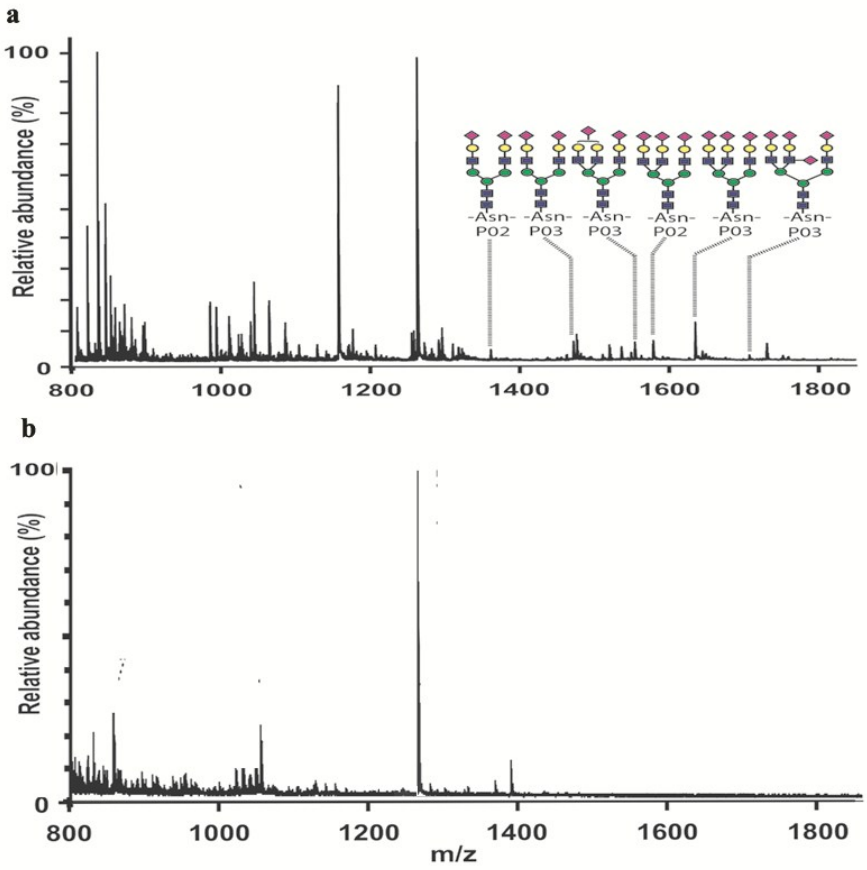


Fig. S2. Mass spectrua of tryptic digests of bovine fetuin before (a) and after loading with 80% CH_3CN/0.1% FA (b). Only several peptides could be observed in the mass spectrum of the flow-through from PolySD-SiO_2, indicating that most of non-glycopeptides and all glycopeptides retained well on the adsorbents under the sample loading condition.

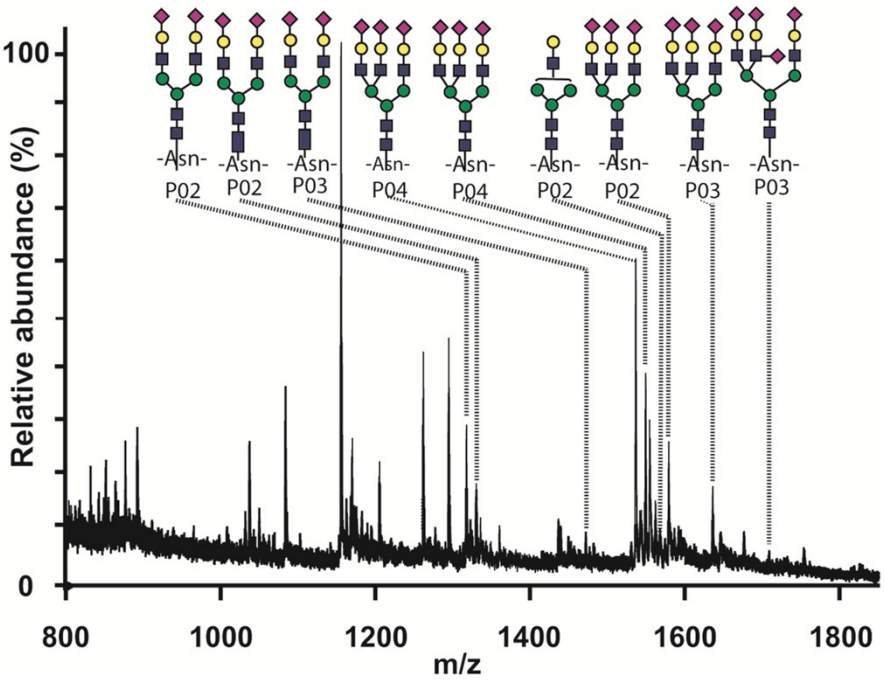


Fig. S3. The mass spectrum of bovine fetuin digests eluted from PolySD-SiO_2 with 40% CH_3CN/H_2O/5 mM NH_4HCO_3 after loading and rinsing with 80% CH_3CN/0.1% FA. This result exhibited that glycopeptides could be eluted from PolySD-SiO_2 with 40% CH_3CN/H_2O/5 mM NH_4HCO_3.

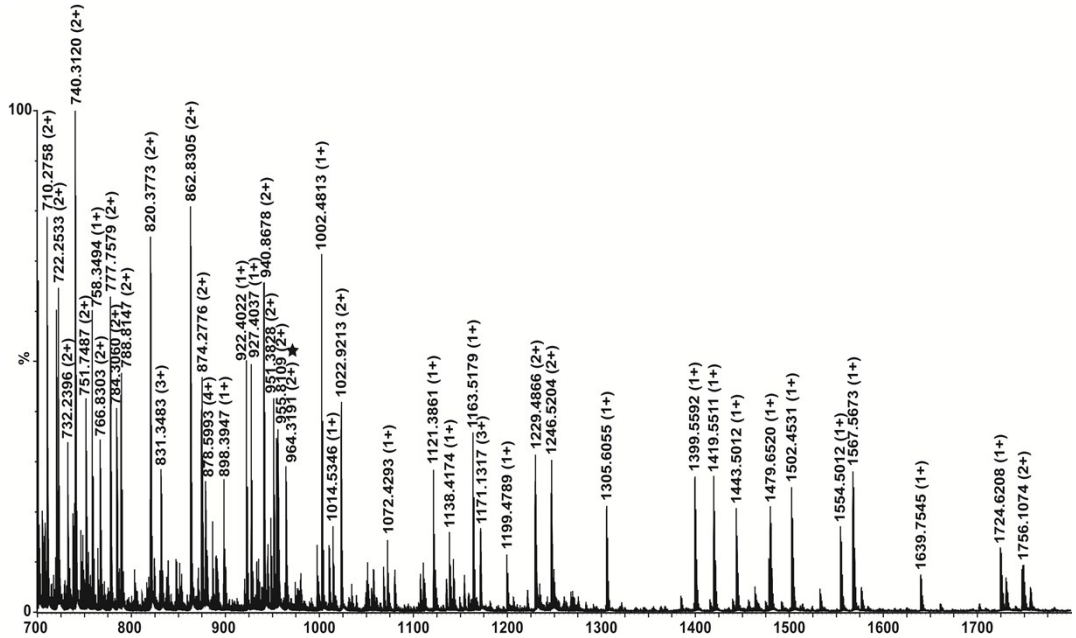


Fig. S4. Mass spectrum of tryptic digests of bovine fetuin and bovine serum albumin (BSA) at molar ratio of 100. Obviously, no glycopeptides could be detected in the mixture of fetuin

and BSA at a molar ration of 1:100.

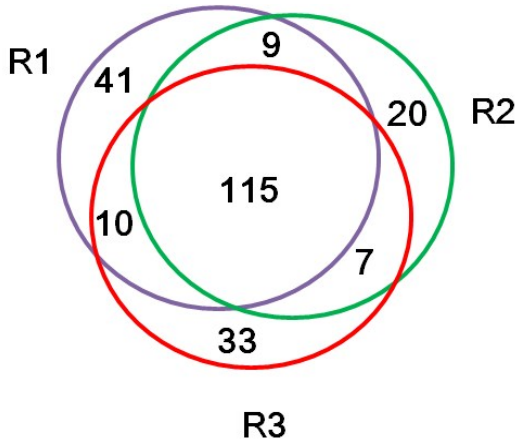


Fig. S5. Venn diagram of the glycosylation sites identified from the HeLa cell lysate after treatment with PolySD-SiO_2 in three technique replicates. This result indicated the high reproducibility of PolySD-SiO_2 based enrichment method .

References

1. F. Wang, R. Chen, J. Zhu, D. Sun, C. Song, Y. Wu, M. Ye, L. Wang and H. Zou, *Anal. Chem.*, 2010, **82**, 3007-3015.
2. J. Cox and M. Mann, *Nat. Biotechnol.*, 2008, **26**, 1367-1372.